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Oxidation of chylomicron remnant-like particles inhibits their uptake by THP-1 macrophages by apolipoprotein E-dependent processes

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Abstract

The influence of the oxidative state of chylomicron remnants (CMR) on the mechanisms of their uptake and induction of lipid accumulation by macrophages derived from the human monocyte cell line, THP-1, during foam cell formation was investigated using chylomicron-remnant-like particles (CRLPs) at 3 different levels of oxidation. The oxidative state of CRLPs was varied by exposure to $CuSO_4$ (oxCRLPs) or incorporation of the antioxidant, probucol (pCRLPs) into the particles. oxCRLPs caused significantly less accumulation of triacylglycerol in the macrophages than CRLPs, and their rate of uptake was lower, while pCRLPs caused more lipid accumulation and were taken up faster. Uptake of all 3 types of particles was inhibited to a similar extent when entry via the low density lipoprotein (LDL) receptor related protein (80–90%), LDL receptor (−30–40%), CD36 (−40%) and phagocytosis (−35–40%) was blocked using lactoferrin, excess LDL, anti-CD36 and cytochalasin D, respectively, but blocking scavenger receptors-A or -B1 using poly inosinic acid or excess HDL had no effect. These findings show that oxidation of CRLPs lowers their rate of uptake and induction of lipid accumulation in macrophages. However, oxidation does not change the main pathways of internalisation of CRLPs into THP-1 macrophages, which occur mainly via the LRP with some contribution from the LDLr, while CD36 and phagocytosis have only a minor role, regardless of the oxidative state of the particles. Thus, the effects of CMR oxidation on foam cell formation contrast sharply with those of LDL oxidation and this may be important in the role of dietary oxidized lipids and antioxidants in modulating atherosclerosis.

Keywords: Chylomicron remnants; Foam cells; Oxidized lipoproteins; Macrophages; Atherosclerosis

1. Introduction

Chylomicron remnants (CMR) carry lipids of dietary origin from the gut to the liver for processing [1] and there is now a large and growing body of evidence indicating that these lipoproteins are strongly atherogenic. They have been shown to be taken up into the artery wall as efficiently as LDL [2–5]; remnant-like particles containing apolipoprotein E (apoE) have been isolated from human aortic intima and atherosclerotic plaque [6,7]; and delayed clearance of CMR from the circulation correlates with lesion development [8,9]. Moreover, we and others have shown that CMR induce extensive lipid accumulation causing foam cell formation in human monocyte-derived macrophages (HMDM) [10] and in human and murine monocyte/macrophages cell lines [11,12].

Low density lipoprotein (LDL) plays a major role in atherogenesis and in foam cell generation, but oxidation of the lipoprotein particles, a process which can occur within the artery wall, is necessary before extensive lipid accumulation is induced [13]. In striking contrast, CMR do not require prior oxidation to cause macrophages to form foam cells [10–12]. However, our studies have demonstrated that incorporation of lipophilic antioxidants into the particles enhances, rather than inhibits, lipid uptake and accumulation in the cells [14,15], suggesting that the oxidative state of CMRs may play a role in their induction of foam cell formation, but in the opposite way to that of LDL. Oxidized CMR could occur either in the artery wall by the action of the cell-associated lipoxygenase and myeloperoxidase which are believed to oxidize LDL, or in the

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circulation, because dietary oxidised lipids, which are produced when fat is cooked at high temperatures, have been shown to be transported in these lipoproteins [16,17]. Clearly, therefore, it is important for the understanding of the atherogenicity of CMR to establish how their oxidation influences their uptake and induction of foam cell formation and the pathways by which CMR are internalised by the cells.

It has been demonstrated that CMR are taken up by the liver by apolipoprotein E (apoE)-dependent pathways mediated by the LDL receptor (LDLr) and the LDL receptor-like protein (LRP) [1]. The exact mechanisms by which CMR are taken up by macrophages, however, are not yet definitively established, and nothing is known about the effects of oxidation of the particles on the routes by which they are internalised. The LDL receptor (LDLr) appears to play a part [1], but as it is down-regulated by the influx of cholesterol into cells, native LDL does not induce foam cell formation [16], and our studies [18] have suggested that the delivery of cholesterol to macrophages by CMR has a similar effect. Thus, other mechanisms are also likely to be involved, and evidence from experiments using antibodies to the LDLr and animals lacking the LDLr supports this view [10]. Candidates include the LRP [19], the apoB48 triacylglycerol-rich lipoprotein receptor (apoB48r) [20], an as yet unidentified 43 kDa protein described by Elsegood et al. [21] and scavenger receptors such as scavenger receptor A (SR-A) and CD36 [1]. Phagocytosis has also been suggested as a possible mechanism [22]. Since the route of uptake of LDL by macrophages is profoundly changed after oxidation from the regulated LDLr to the unregulated scavenger receptors [16], it is important to establish whether the oxidative state also alters the mechanisms of uptake of CMR by the cells.

The aim of this study is to investigate the effects of the oxidative state of CMR on their uptake by macrophages and on the accumulation of lipid within the cells, and to determine how oxidation affects the pathways involved in the internalisation of the particles. Chylomicron remnant-like particles (CRLPs) at three different levels of oxidation (CRLPs, oxidized CRLPs (oxCRLPs) and CRLPs containing the antioxidant probucol (pCRLPs)) and macrophages derived from the human monocyte cell line THP-1 were used as the experimental model, and the mechanisms of uptake were evaluated using specific inhibitors of the processes believed to be involved. The findings clearly demonstrate that oxidation of CRLPs reduces the rate of their uptake by THP-1 macrophages and decreases lipid accumulation in the cells, and further show that this is due to differential interaction with apoE dependent receptors.

2. Materials and methods

RPMI 1640 medium, fetal bovine serum (FBS), L-alanyl-L-glutamine (glutamax) penicillin/streptomycin and β-mercaptoethanol were obtained from Gibco (Paisley, UK). FBS was heat inactivated (56 °C, 30 min) before use. Trypan blue, fatty acid-free bovine serum albumin (BSA), phospholipids, cholesterol, cholesteryl oleate, phorbol 12-myristate 13-acetate (PMA), Oil red O, probucol, poly inosinic acid (poly I), lactoferrin and cytochalasin D were supplied by Sigma (Poole, UK). 1′1′-dioctadecyl-3,3-3′,3′-tetramethylindocarbocyanide perchlorate (DiI) was from Cambridge Bioscience (Cambridge, UK). The blocking antibody for CD36 was obtained from Immunodiagnostic Systems Ltd (Tyne and Wear, UK) and cholesterol oxidase from Merck Biosciences Ltd (Nottingham, UK). DiI-labelled acetylated LDL (acLDL) was purchased from Molecular Probes (Paisley, UK).

2.1. Preparation of lipoproteins

LDL and high density lipoprotein (HDL) were isolated from human plasma (National Blood Service, London UK) by ultracentrifugation. Plasma was layered under 0.9% NaCl (d=1.006 g/ml), centrifuged for 5 h at 100,000×g (4 °C), and the top fraction discarded. The density of the bottom layer was raised to 1.063 g/ml with KBr, layered under KBr (d=1.063 g/ml) and centrifuged for 16 h at 175,000×g (4 °C). LDL was collected from the top fraction by tube slicing. For the preparation of HDL, the bottom fraction was then adjusted to d 1.21 g/ml with KBr, layered under KBr (d 1.21 g/ml) and centrifuged at 175,000×g/ml at 4 °C for 18 h and the top fraction containing HDL was collected by tube slicing. LDL and HDL were dialysed against saline for 48–72 h prior to used.

CRLPs were prepared by sonication (power setting 22–24 μm, 20 min, at 56 °C) of a lipid mixture containing 70% trilinolein, 2% cholesterol, 3% cholesteryl ester and 25% phospholipids in 0.9% NaCl (w:v) in Tricine Buffer (20 mM, pH 7.4) followed by stepwise density gradient (2.5 ml d 1.065 g/ml, 2.5 ml d 1.020 g/ml, 3 ml d 1.006 g/ml) ultracentrifugation as described by Diard et al. [23] at 17,000×g for 20 min at 20 °C. The upper layer of grossly emulsified lipids was then removed and replaced with an equal volume of NaCl solution (d=1.020 g/ml) and tubes were centrifuged at 70,000×g for 1 h (20 °C). For apoE binding, lipid particles collected from the top layer were incubated with the dialysed d 1.063–1.21 g/ml fraction of human plasma (National Blood Transfusion Service, North London Centre, UK) prepared as described above at 37 °C with shaking for 4 h (1 volume of particles: 2 volumes plasma). The CRLPs containing apoE were then isolated by ultracentrifugation at d=1.006×g/ml (120,000×g for 12 h at 4 °C), harvested from the top layer, purified by a second centrifugation at the same density (202,000×g for 4 h at 4 °C) and stored at 4 °C under argon until required. All preparations were used within 1 week. For DiI-labelled CRLPs and pCRLPs, probucol (1 mg) and/or DiI were added to the lipid mixture prior to sonication. CRLPs were oxidized by incubation with $CuSO_4$ (20 μM) with shaking for 5 h at 37 °C and the $CuSO_4$ was then removed by dialysis (0.9% NaCl, 24 h, 4 °C). The oxidation process had no effects on the fluorescent properties of the DiI label.

2.2. Culture of THP-1 cells

THP-1 monocytes were maintained in RPMI 1650 culture medium containing 10% (v/v) FBS, glutamax (2 mM), penicillin/streptomycin (100 U/ml/100 μg/ml) and β-mercaptoethanol (20 μM) (culture medium). The cells were differentiated into macrophages by incubation with PMA (200 ng/ml) for 72 h at 37 °C in 5% CO_2/95% air. The medium containing PMA and any non-adherent cells were then removed and the macrophages were washed with PBS (3×1 ml) and incubated with CRLPs or DiI-labelled CRLPs (30 μg cholesterol/ml) in the presence or absence of specific inhibitors as indicated in the text. The inhibitors were added 1 h prior to the addition of the CRLPs. Cell viability as assessed by Trypan blue exclusion was >95% and was not affected by any of the CRLP types or conditions used. After the incubation, the macrophages were washed (culture medium 3×1 ml) and lipid accumulation was assessed by staining with Oil red O, or harvested for lipid analysis [24]. For studies with DiI-labelled CRLPs, the fluorescence associated with the cells was assessed by viewing with a Zeiss LMS 510 laser -scanning confocal microscope and quantified by absorbance volume analysis, or by fluorescence-activated cell analysis (FACS) using a BD FACS Calibur flow cytometer (BD Biosciences, Oxford, UK). For FACS analysis, cells were harvested in PBS containing EDTA (5 mM) and lidocaine -HCl (8 mg/ml), incubated for 20 min at 37 °C and centrifuged at 10,000×g (2 min). The cell pellet was then resuspended and fixed in PBS containing 4% formalin.

2.3. Analytical methods

For mRNA analysis, total RNA was extracted from THP-1 macrophages before or after incubation with CRLPs (30 μg cholesterol/ml) using a kit from Sigma (Poole, UK) and first strand synthesis was carried out using a kit supplied by Promega (Southampton, UK) according to the manufacturer's instructions.